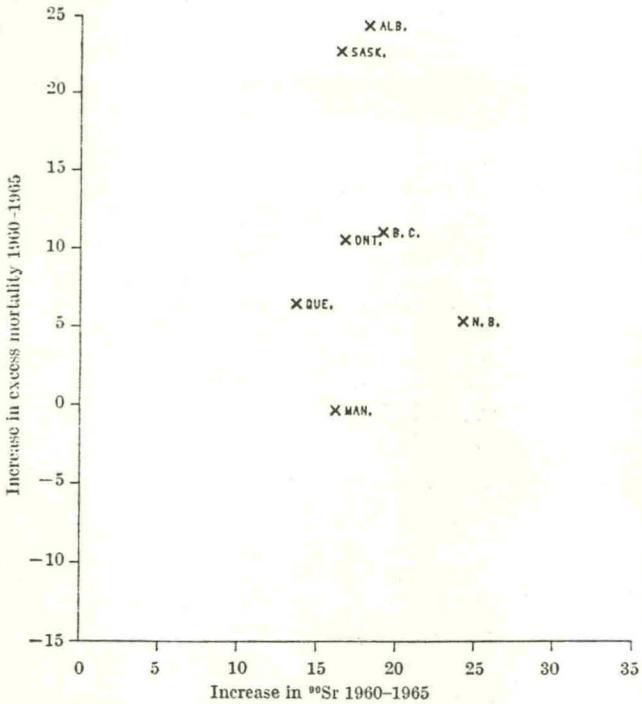

Fig. 2. Change in infant mortality and change in strontium-90 level, calculated using 1946–55 as the basis for the least-squares line. (Least-squares estimate of slope is $b = -0{\cdot}12$.)

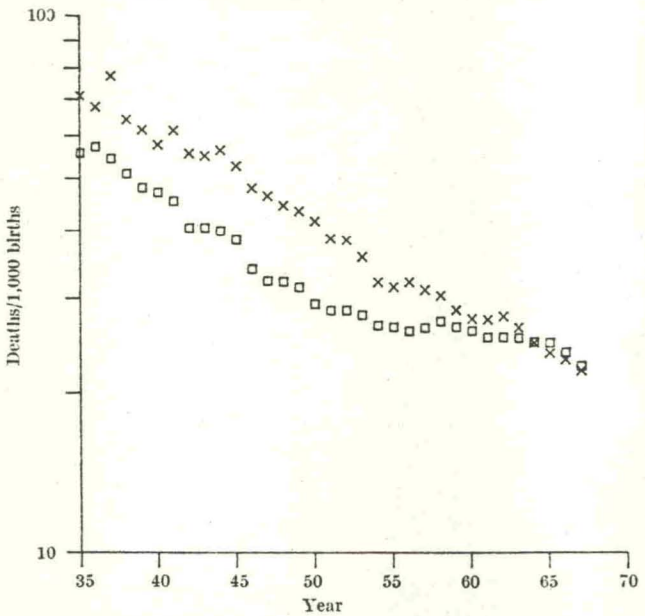

Fig. 3. Infant mortality in Canada and the US. ×, Canada; □, US.

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R. F. Shaw
A. P. Smith

Department of Preventive Medicine and Paediatrics,
Dalhousie University,
Halifax, Nova Scotia.

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Influence of Hydrostatic Pressure on the Reversible Polymerization of Fibrin Monomers

The conversion of fibrinogen to fibrin, which is the last stage of the complex process of blood coagulation, is composed of three successive reactions[1]: an enzymatic hydrolysis by thrombin of four arginyl-lysyl bonds per molecule of fibrinogen, yielding a fibrin monomer; a spontaneous polymerization of monomers to form a gel or fibrin clot; and an enzymatic transamidation by fibrin stabilizing factor (factor XIII or fibrinase) between ε-amino groups of lysyl and glutaminyl side chains. The mechanism of the reversible polymerization of fibrin monomers remains largely unknown, although it can be studied separately because fibrin monomers can be stabilized in acidic media of high ionic strength such as 1 M NaBr, *p*H 5·3[2].

The hypothesis of hydrogen bonding between ionizable groups, in which histidyl functions as acceptor and tyrosyl or lysyl as donors, as proposed by Sturtevant *et al.*[3], accounts for the depolymerizing effect of dilution, of slight temperature increase and of salts and urea. It also explains the *p*H range of polymerization (between 5·5 and 10·5), the *p*H evolution during the reaction (proton liberation below *p*H 7·5) and the inhibiting effect of chemical blocking of histidyl and tyrosyl residues. The only real shortcoming of this hypothesis is its failure to account for the magnitude of the heat evolution during polymerization[4]. It seems that additional heat evolving reactions such as hydrogen bonding between non-ionizable groups, ion pair bonding, hydrophobic bonds or conformational changes should also be involved. Up to now, however, no sufficient independent evidence for either of those additional reactions has been presented.

The origin of the heat evolution in reversible fibrin polymerization has become an interesting problem since a number of other cases of exothermic protein polymerizations, including flagellin, β-lactoglobulin and lactic dehydrogenase, have been discovered.

A study of the influence of hydrostatic pressure on the polymerization equilibria gives the sign and magnitude of the volume increment, which is dependent on the nature and the number of the interactions involved in polymerization. Volume changes during polymerization have been noted before, for example in tobacco mosaic virus protein[5] and in myosin[6].

Bovine fibrinogen was prepared according to the method of Blombäck and Blombäck[7]; fibrin monomers were prepared according to Donnelly[2] as modified by Endres[4].

A technique for observing association and aggregation reactions of proteins by means of light scattering measurements under high pressure has recently been developed by Putzeys and Vaneghem (unpublished thesis). We were able to use the apparatus and technique in the Laboratory of Biochemistry I. Light scattering measurements were taken at 90° and under pressure up to 3,200 kg/cm². Temperature variations resulting from application of pressure were prevented by a water jacket, maintained at 25° C.

Fibrinogen and fibrin monomer solutions showed no change in light scattering under pressure up to 3.200 kg/cm² except for a small stepwise change during the application of pressure, caused by deformation of the windows in the optical path (Fig. 1A).

Intermediately polymerized fibrin monomer solutions of 10 mg/ml. in 1 M NaBr, 0·05 M acetate buffer, with a *p*H varying from 5·75 to 6·15, showed a large decrease in light scattering under pressure (Fig. 1B–E) and were apparently already completely depolymerized under a pressure of 2,500 kg/cm², for after equilibration a further increase did not change the amount of scattered light. The polymerization–depolymerization reaction was always completely reversible. The influence of the concentration